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SIZE OF NEW-BORN LARVAE OF COCKROACHES INCUBATING EGGS INTERNALLY

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Abstract—New-born larvae from small litters of *Diploptera punctata* tend to be larger than individuals from large broods. This relationship between embryo size and size of litter does not hold for *Nauphoeta cinerea* and *Leucophaea maderae*. The correlation, or lack of it, can be explained on the basis of nutrition of the embryo. *Diploptera* embryos derive nourishment and water from the mother; in small litters a greater amount of nutritive material presumably is made available to the developing embryos in the uterus. In *Nauphoeta* and *Leucophaea* there is no competition for nutritive material because the embryos in the brood sac derive only water from the mother during gestation.

INTRODUCTION

AMONG vertebrate multiparous animals, the production of an abnormally small litter may result in oversized foetuses (BENESCH and WRIGHT, 1951). In normally uniparous animals, individuals resulting from multiple births are smaller than single offspring; in bovines the reduced weight of an individual twin is due to a reduced placenta area or a reduced amount of available nutrients for each foetus, and to the shortened gestation period (in ROBERTS, 1956).

Some cockroaches incubate their eggs internally in a brood sac or uterus (ROTH and WILLIS, 1954, 1958b). The eggs of practically all of these species, as well as cockroaches that deposit oöthecae outside the body, increase in water content and lose solids during embryogenesis (ROTH and WILLIS, 1955a–c, 1957, 1958a, b). However, in *Diploptera punctata* (Eschscholtz) the embryos increase in both water and solids during their confinement in the uterus (ROTH and WILLIS, 1955d).

Well-developed embryos of *Diploptera* may differ considerably in size, in the same oötheca, even though the eggs when oviposited are all about the same length. HAGAN (1951) noted this difference in size of individuals in the uterus but believed that it occurred only when there were thirteen or fourteen eggs in the oötheca and that the 'extra' embryos were small and '. . . apparently failed to mature'. Hagan suggested that '. . . such accessory embryos develop in the second oöcytes, which have, somehow, been passed down with the mature ones through some functional disturbance. In another instance an oötheca with twelve eggs had embryos of the same age but one was considerably larger than the others. Perhaps it failed to pass into the oviduct to join an earlier brood.'

Variation in size of new-born larvae may occur normally in all litters (e.g. Fig. 3, litter of thirteen), regardless of the number of eggs initially placed in the oötheca. HAGAN (1951) suggested that the right colleterial gland of *Diploptera* might supply the nourishment for the embryos. However, STAY and ROTH (1962) showed that the removal of either or both colleterial glands did not prevent the development of the embryos in the uterus. In *Diploptera* the pleuropodia are intimately associated with the uterus, and HAGAN (1939, 1941, 1951) suggested that the pleuropodium may function in the transfer of liquids (or of both liquids or gases simultaneously) from the mother to the embryos. We noted on several occasions that unusually small embryos in the uterus were not properly oriented in the oötheca. These embryos would be squeezed between adjacent embryos so that they were not in intimate contact with the uterine wall; one unusually small embryo was rotated so that its ventral (rather than dorsal) surface was in contact with the uterus. It is possible that unusually small embryos in *Diploptera* result from improper contact between the organs (pleuropodia ?) responsible for the transfer of nutritive material through the mother's uterus.

It seems likely that *Diploptera* embryos in the uterus compete for nutritive material and perhaps for space as well. To determine if this is so, the effect of artificially reducing the number of eggs in the oötheca on the size of the larvae when born was investigated. For comparative purposes, a similar experiment was performed on *Nauphoeta cinerea* (Olivier) and *Leucophaea maderae* (Fabricius). The eggs of these cockroaches are also incubated internally, but they increase only in water content, and lose dry weight during gestation (ROTH and WILLIS, 1955c).

MATERIALS AND METHODS

The cockroaches were reared on Purina laboratory chow at 27 ± 1°C. The average number of eggs in the oötheca of *Nauphoeta* is 32·8 (WILLIS *et al.*, 1958). Oviposition may take more than 2 hr (ROTH and WILLIS, 1954). To reduce the size of the litter, various numbers of eggs were removed from females as oöthecae were being formed and the eggs were still oriented with their axes vertically (see Pl. 12, Fig. 86, in ROTH and WILLIS, 1954); the females deposited the remaining eggs in the uterus.

The average number of eggs per oötheca in *Leucophaea* is 33·8; however, the number born may be considerably less and WILLIS *et al.* (1958) recorded the average number hatching as 18·3. SCHARRER (1958) found the number of larvae born per litter to range from 18 to 42 with a mean of 32·3 which is about the average number of eggs per oötheca. In the present experiments, the number of eggs in the öothecae of *Leucophaea* was not reduced experimentally. Small litters were the result of some eggs not developing or maturing; it is not known if partly developed eggs influenced the size of the individuals that hatched normally.

In *Diploptera*, oviposition may take only a few minutes (ROTH and WILLIS, 1955d) and it is easily overlooked because only one or two eggs extend out beyond the end of the abdomen (see Pl. 28, Fig. 38, in ROTH and WILLIS, 1958b). The following techniques were employed, without success, to reduce the number of uterine eggs in this species: (1) some eggs were cut away from the oötheca within a day after oviposition; (2) several eggs were crushed but were allowed to remain in the uterus; (3) some eggs were removed while the female was ovipositing. The only successful experimental method of reducing the size of the litter was to remove one ovary. As in *Leucophaea maderae* (SCHARRER, 1958), removing one ovary from *Diploptera* results in half the normal number of eggs being oviposited. As in *Leucophaea*, some eggs in *Diploptera* and *Nauphoeta* may fail to develop so that litters are obtained with fewer individuals than there were eggs laid.

The larvae were preserved in 70% alcohol within a day after parturition. The widths of the head and pronotum at their widest points were measured from the dorsal surface using an ocular micrometer and dissecting microscope. No significant difference was found between male and female larvae from the same litter (in large series of litters) so that the measurements of both sexes were combined.

RESULTS AND DISCUSSION

One hundred pregnant *Diploptera*, with well-developed embryos in the uterus, were taken at random from cultures. The embryos were removed, counted, and

TABLE 1—NUMBER OF OVARIOLES AND NUMBER OF EGGS PRESENT IN THE UTERUS OF PREGNANT *Diploptera punctata*

Total number of ovarioles	Number of eggs in uterus			Number of females
	Developed	Undeveloped or dead	Total	
11	9	2	11	1
12	7	4	11	2
12	8	2	10	1
12	8	4	12	2
12	9	0	9	1
12	9	3	12	4
12	10	0	10	1
12	10	1	11	2
12	10	2	12	7
12	11	0	11	3
12	11	1	12	18
12	12	0	12	31
13	6	7	13	1
13	9	4	13	1
13	10	3	13	2
13	11	1	12	2
13	11	2	13	3
13	12	1	13	5
13	13	0	13	8
14	9	5	14	1
14	11	2	13	2
14	12	2	14	1
14	13	1	14	1

checked for development; the ovaries of each female were removed and ovarioles counted and compared with the number of embryos in the uterus. The results are shown in Table 1. Only one female had fewer than 12 ovarioles. Seventy-two per cent had 12, 22 per cent 13, and 5 per cent 14 ovarioles. Fourteen females had fewer embryos in the uterus than ovarioles in the ovary. The number of developed eggs in the uterus is indicative of the number of individuals a female may normally give birth to in a litter and this may vary from 6 to 13. The average number of eggs which hatch is 9·8 (Willis *et al.*, 1958). None of the females had more

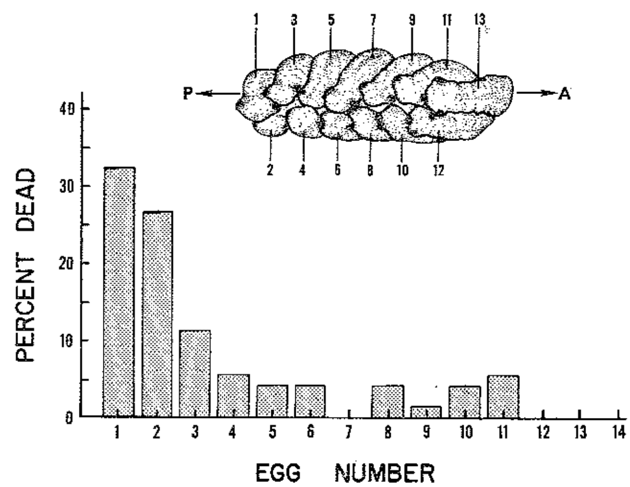

Fig. 1. Histogram showing the frequency distribution of undeveloped eggs or dead embryos in the uterus of *Diploptera*. The drawing at the top represents an oötheca containing thirteen well-developed embryos which are numbered. The egg numbers indicated on the axis of abscissas refer to the numbers of the embryos in the drawing. A and P indicate the anterior and posterior parts of the uterus, respectively. The heads of the embryos lie facing the left side of the female, and all odd numbered embryos lie on the ventral surface of the uterus.

embryos in the uterus than ovarioles; those that oviposit 13 or 14 eggs have 13 or 14 ovarioles and the 'supernumerary' eggs are not, as Hagan (1951) suggested, due to the development of the second oöcyte of one of the ovarioles. Litters with less than 12 individuals are usually due to the fact that some of the eggs in the uterus do not develop or develop partly, but die.

The oötheca lies in the uterus with the micropylar (cephalic) ends of the eggs facing the left side of the female. The eggs closest to the genital opening are the first to pass down from the ovaries during oviposition. When the oötheca is retracted the last laid eggs come to lie in the anterior end of the uterus. The eggs lie in two rows except for one or sometimes two of the last laid eggs which may face the double row. Data were obtained on the position of the eggs of *Diploptera* which failed to develop or died after some development occurred. The embryos were numbered as follows; the ventral embryo lying close to the genital opening was 1, the embryos above and next to it, 2 and 3, respectively, and so on (Fig. 1). Forty-one oöthecae containing one or more undeveloped eggs or dead embryos were examined. The frequency distribution of the positions of these undeveloped eggs or dead embryos is shown in Fig. 1. Fifty-nine per cent of eggs numbers 1 and

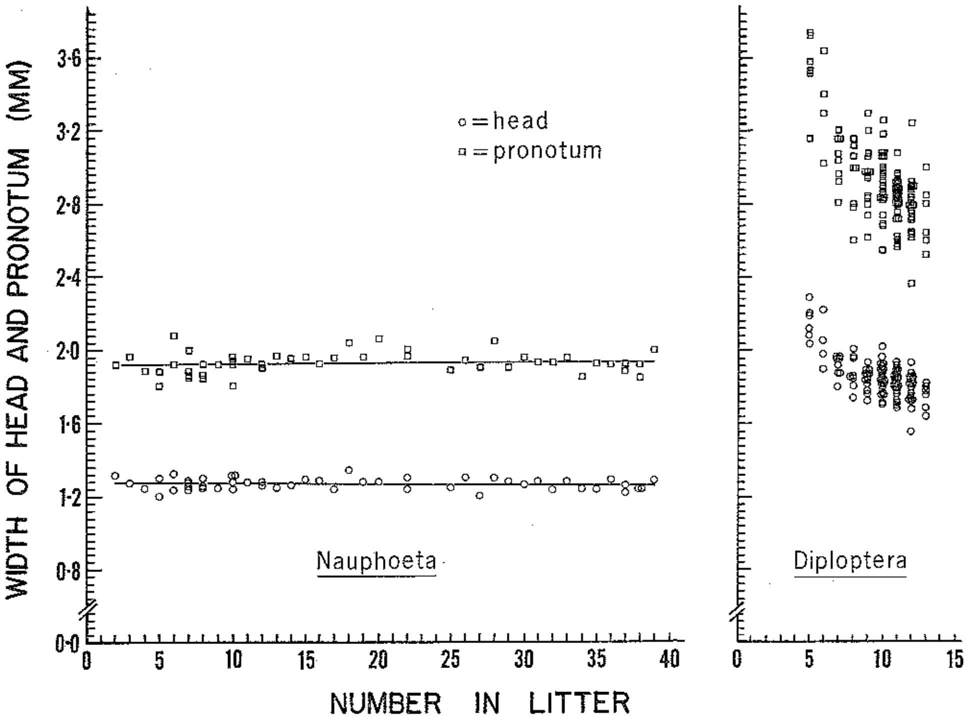

FIG. 2. Relationship between size of brood and size of new-born individuals in *Diploptera punctata* and *Nauphoeta cinerea*. Each pair of symbols represents the means of measurements of larvae from a single litter (114 litters for *Diploptera* and 49 for *Nauphoeta*). In *Diploptera* the litters containing five individuals and two litters containing six larvae were obtained by removing one ovary from the females.

Regression lines and correlation coefficients were calculated for *Nauphoeta* as follows:

$$\text{Head: } Y = 1{\cdot}277 - 0{\cdot}000516X \ (r = 0{\cdot}14).$$
$$\text{Pronotum: } Y = 1{\cdot}922 + 0{\cdot}000313X \ (r = 0{\cdot}06).$$

2 (those laid first and closest to the genital opening) failed to develop or eventually died. Although some of these eggs may not have been fertilized, another explanation may account for this high mortality of the first laid eggs. The oöthecae of cockroaches that retract eggs into a brood sac do not prevent desiccation of the eggs if they are exposed to the atmosphere (ROTH and WILLIS, 1955c). The newly laid eggs of *Diploptera* are so small and few in number that only the first and second laid eggs may slightly protrude beyond the end of the abdomen (ROTH and WILLIS, 1955d).

The oötheca is thin and only partly surrounds the eggs (ROTH and WILLIS, 1958b, Pl. 29, Figs. 53, 54), even at the time of oviposition (HAGAN, 1951). Although rotation and retraction of the eggs may take only a few minutes in *Diploptera*, it is possible that the brief exposure of the first and second eggs to the atmosphere may kill or prevent them from completing development.

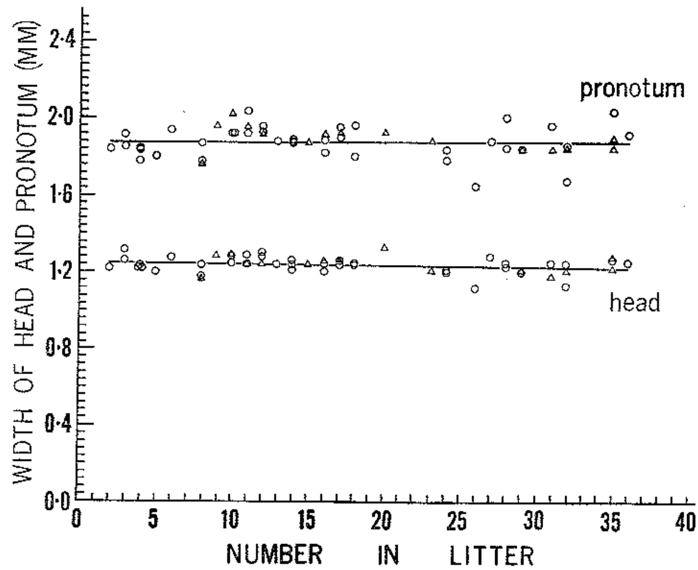

FIG. 4. Effect of starvation during gestation on size of larvae of *Nauphoeta* from different-sized broods. Each symbol (for head and pronotum) represents the mean widths of individuals from a single litter.

Circles = offspring from females without food or water (except for 24 hr) during pregnancy.

Triangles = offspring from females given water *ad libidum* but without food (except for 24 hr) during pregnancy.

Regression lines and correlation coefficients were calculated as follows for larvae represented by circles only:

Head: $Y = 1{\cdot}249 - 0{\cdot}000925X$ $(r = -0{\cdot}02)$.

Pronotum: $Y = 1{\cdot}875 + 0{\cdot}000041X$ $(r = 0{\cdot}005)$.

The relationship between the size of the litter and size of the embryos (as indicated by larvae less than 24 hr old) for *Diploptera* and *Nauphoeta* is shown in Fig. 2 and for *Leucophaea* in Fig. 5. In *Diploptera*, the smaller the litter, the larger the larvae. With fewer eggs in the uterus, a greater amount of nutritive material presumably is made available to the developing embryos. However, in *Nauphoeta* and *Leucophaea* the size of the larvae remains fairly constant regardless of the number incubated in the uterus; the correlation coefficients (r) for head and pronotum were not significant at the 1 per cent level. This is consistent with the fact that the embryos of these species obtain only water from the mother during gestation. Increasing the amount of available space in the uterus has no effect on the size of

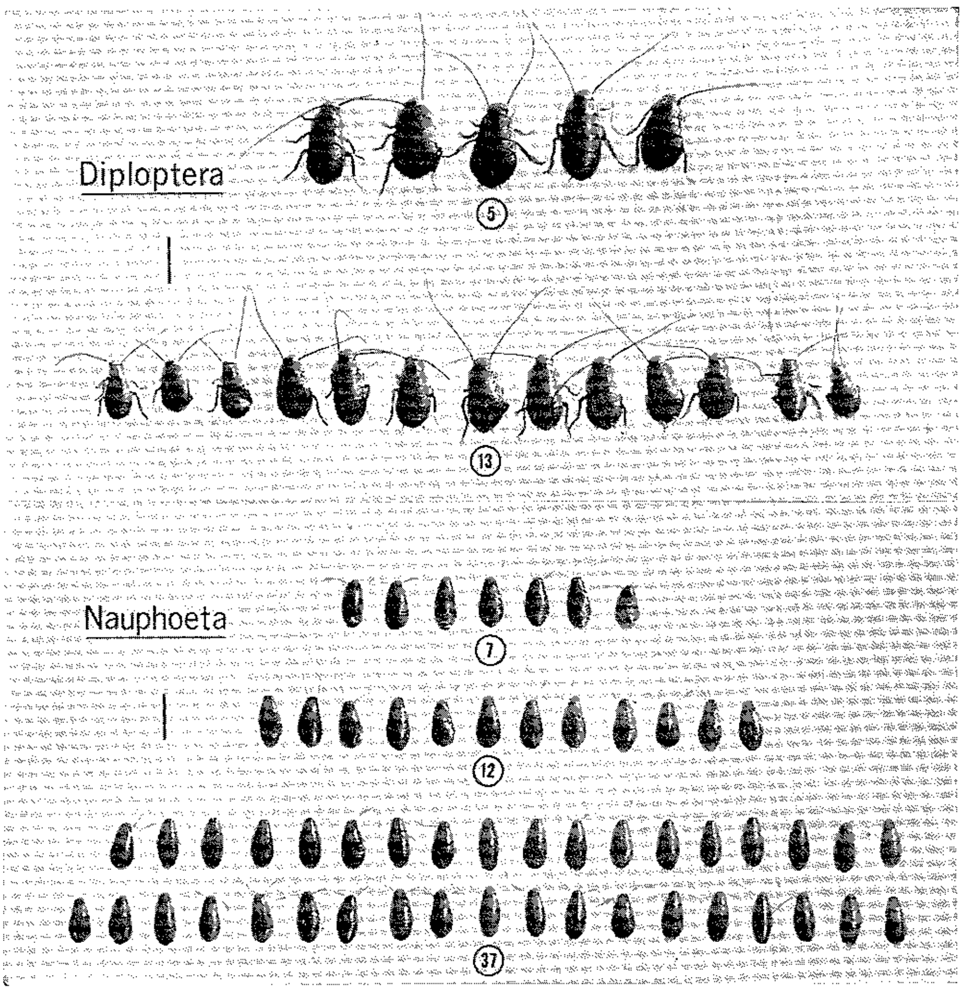

FIG. 3. Larvae of *Diploptera* and *Nauphoeta* from litters containing different numbers of individuals. All of the specimens were preserved within 24 hr after birth. Numbers in circles under the rows of larvae indicate the number of individuals in the litter. Vertical bars = 5 mm.

the embyros in *Nauphoeta*. In all three species the gestation periods differed but were similar for broods of various sizes within the species.

Unmated *Nàuphoeta* females live about 1 month without food and water and about 2 months with water but no food (Willis and Lewis, 1957). One experiment was performed to determine the effect, if any, on the size of larvae born to females that were starved for almost the entire gestation period. Within a day after oviposition, females were isolated from food. One group was without food and water, except for 24 hr only on the sixteenth day of pregnancy, for the entire gestation period. The second group had water *ad libidum* but had access to food 24 hr only on the sixteenth day of pregnancy. The results are shown in Fig. 4.

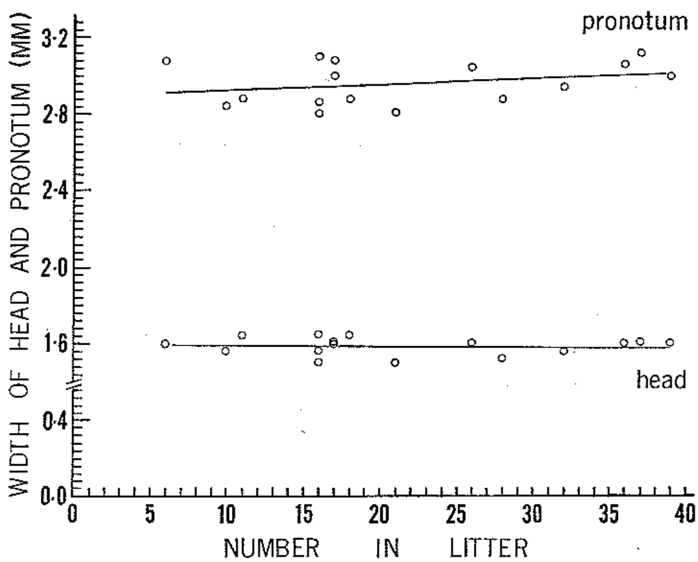

Fig. 5. Relationship between size of brood and size of new-born larvae in *Leucophaea maderae*. Each pair of symbols represents the means of measurements of larvae from a single litter.

Regression lines and correlation coefficients were calculated as follows:

Head: $Y = 1{\cdot}578 - 0{\cdot}000301X$ ($r = -0{\cdot}01$).

Pronotum: $Y = 2{\cdot}892 + 0{\cdot}003151X$ ($r = 0{\cdot}002$).

Females deprived of drinking water have sufficient body water available for normal-sized litters to develop. Regardless of litter size, neither starvation nor starvation and desiccation had any effect on the size of larvae. The correlation coefficients (r) for females starved without water were not significant at the 1 per cent level. However, new-born larvae ($\bar{x}$ head width $= 1{\cdot}234 \pm 0{\cdot}0064$ mm; $\bar{x}$ pronotal width $= 1{\cdot}876 \pm 0{\cdot}0134$ mm) from females starved without water were slightly, but significantly ($P < 0{\cdot}01$) smaller than larvae (head width $= 1{\cdot}268 \pm 0{\cdot}0043$ mm; pronotal width $= 1{\cdot}928 \pm 0{\cdot}0067$ mm) from females that had food and water continuously during gestation.

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